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Hermann-Bank, Marie Louise; Mølbak, Lars

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A NEW PHYLOGENETIC APPROACH TO INVESTIGATE THE INTESTINAL BACTERIAL COMPOSITION

M.L.H. Hermann, L. Mølbak

National Veterinary Institute, Technical University of Denmark, Copenhagen, Denmark

A new type of diarrhoea in neonatal piglets, called NNPD, has arisen in Europe. Which role the intestinal microbiota plays in NNPD is unknown. Several methods can be applied to compare faecal samples from sick and healthy animals; in this study we used Real-Time PCR combined with next generation sequencing.

The objective of this study is to demonstrate the applicability of the Access Array 48.48 (AA48.48) from Fluidigm® in investigating the composition of the intestinal microbiota. The array makes it possible to analyse 48 primer pairs against 48 samples resulting in 2304 individual Real-Time PCR reactions in a few hours. Subsequently the 16S rDNA amplicons can be harvested and used for next generation sequencing.

The experimental setup was designed so that the primers represented a phylogenetic overview of the biggest groups of intestinal bacteria. Primers were mainly chosen from published literature, meaning their use had already been verified. DNA from reference bacteria and DNA from faecal samples were used to check specificity and sensitivity of the assay when analysing bacterial communities.

The heat map showed nice dilution colour series consistent with the diluted sample concentrations ranging from 50 to 0.005 ng/µl and in agreement to the expected results. This implies that the AA48.48 is a useful tool when examining the bacterial composition of faeces and maybe superior to competitive methods, as it gives a quantitative measurement of the bacteria in the sample and afterwards it is possible to get further phylogenetic information, as the DNA can be harvested and sequenced.